A comparative study of the abdominal tympanal organs in Pyralidae (Lepidoptera)

I. Description, terminology, preparation technique

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Abstract

Our present knowledge on abdominal tympanal organs is briefly reviewed. A latinized nomenclature is proposed for those sclerotized parts that are useful in taxonomy. A preparation technique that preserves and allows display of the tympanal organs is described. A standardisation is suggested.

Introduction

Although abdominal tympanal organs were noticed quite early by various authors (Sharp, 1889; Petersen, 1904a, 1904b; Kennel, 1912; Jordan, 1905; Eltringham, 1924) and even used in systematic works (Börner, 1925; Handlirsch, 1925) no complete morphological study was available until the work of Kennel & Eggers (1923) was published. They studied these organs in the Geometridae, Pyralidae, Cymatophoridae and Uraniidae.

Later, starting in the fifties, numerous papers on tympanal organs were published, emphasizing ethological and physiological aspects (Treat, 1955; Belton & Kempster, 1962; Belton, 1962; Hertveldt, Moerman, Gillard, 1969; Agee, 1969; a.o.). Only a few papers on the morphology of abdominal tympanal organs were published (Mullen & Tsao, 1971a, 1971b; Coro, 1972).

BERGER (1957) described abdominal tympanal organs in the genus *Dudgeonea* HAMPSON, a former representative of the Cossidae, for which he erected the family Dudgeonidae.

Tympanal organs as a diagnostic feature in the Pyralidae were only used by Marion (1954) and Munroe (1972, 1976). But their views on the value of these structures do not reach further than those of Guenée (1854) and Börner (1925).

MINET (1981, 1983) further elaborated KENNEL & EGGERS (1933) paper on the basis of modern techniques and literature published since then. His phylogenetic approach, starting from the higher levels of the taxonomic hierarchy and working downwards leads to speculative generalisations.

Description

General

The paired tympanal organs of the Pyralidae are located on the anterior part of the first (second according to BÖRNER, 1925 and BROCK, 1971) abdominal sternite. Principally they are composed of a thin cuticular membrane, the tympanum, associated with an air sac and chordotonal sensilla. The air sac of each organ is on the inside limited by tracheal epithelium and lodged in a cavity formed by an invagination of its segment. The air sacs touch anteriorly the median thoraco-abdominal air sac. The latter is not associated with chordotonal sensilla but is thought to delimit the contra-tympana of the metathorax (KENNEL & EGGERS, 1933; MINET, 1983).

The tympanum itself is very thin in its transparent zone. It seems to be formed by a flattened layer of epidermis that becomes very irregular in some parts. It is connected medially with the conjunctivum which is white and opaque when dry. Histologically it consists of a thin chitinous layer lined inside by a thin epidermal layer (Coro, 1972).

According to the tympanum-conjunctivum connection in the transversal plane two major groups can be recognized in the Pyralidae (nomenclature according to FLETCHER & Nye (1984)).

- 1) tympanum and conjunctivum are in the same plane: type 'Pyralidae' (BÖRNER, 1925; HANNEMANN, 1964; MINET, 1981): Galleriinae, Epipaschinae, Chrysauginae, Pyralinae, Phycitinae and Peoriinae.
- 2) tympanum and conjunctivum make an angle: type 'Crambidae' (BÖRNER, 1925; HANNEMANN, 1964; MINET, 1981): Crambinae, Midilinae, Nymphulinae, Scopariinae, Schoenobiinae, Cybalomiinae, Linostinae, Evergestinae, Odontiinae, Glaphyriinae and Pyraustinae.

This subdivision seems to coincide with two types of tympanal organs (Kennel & Eggers, 1933). Minet (1981, 1984) includes also the Dudgeonidae. But due to the presence of abdominal tympanal organs in some tineids (G. S. Robinson, pers. comm.) it seems more probable that these organs evolved separately in different groups.

The purpose of this paper is to give a brief description of the structure of the abdominal tympanal organs of the Pyralidae s.s. in order to achieve a uniform

latinized nomenclature. Only the integumental structures of the abdomen are considered here since these give the most useful systematic characters at the lower taxonomic levels. For the detailed histological structures of these organs see Kennel & Eggers (1933), Coro (1972) and Minet (1983).

Terminology (Pl. 1 & 2)

The names between brackets are the french alternatives proposed by MINET (1981).

bulla tympani, pl: bullae tympani (caisse tympanique): formed by an invagination of the first-sternite; usually bean-shaped, the longitudinal axis parallel with the body axis.

Two forms are recognized:

- 1) open: the tracheal membrane is not completely surrounded by the bullae tympani, restricted to the 'Crambidae' type.
- 2) closed: the tracheal membrane is completely surrounded, restricted to the 'Pyralidae' type.

conjunctivum (conjonctivum): membrane immediately connected with the tympanum, opaque when dry.

fornix tympani (cadre tympanique) : framework which supports the tympanum.

intersegmental thoraco-abdominal membrane: intersegmental membrane between the metathorax and the first abdominal sclerites. In the ventral region it can be expanded forming a torulus tympani or a praecinctorium.

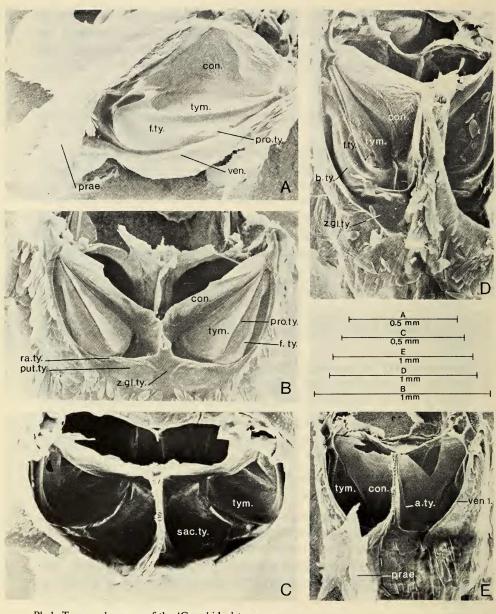
ligna tympani (ligne tympanique): an imaginative line between the conjunctivum and the tympanum. Comparing the fornix tympani with a bow then the ligna tympani is the string.

paraspina (paraspina): needle-like sclerification of the posterior part of the ligna tympani. Common in the organs of the 'Pyralidae' type.

pons tympani: the median zone of the first sternite forming a connection between the praecinctorium or torulus tympani with that sternite. This zone is constricted to the vertical part of the connection, the horizontal bars are the rami tympani (sing.: ramus tympani). The alae tympani (sing.: ala tympani) connect the pons tympani with the posterior end of the fornix tympani.

praecinctorium (praecinctorium): median expansion of the intersegmental thoraco-abdominal membrane.

In the 'Pyralidae' type it is usually very simple: torulus tympani (bourrelet ventral).



Pl. 1. Tympanal organs of the 'Crambidae' type.

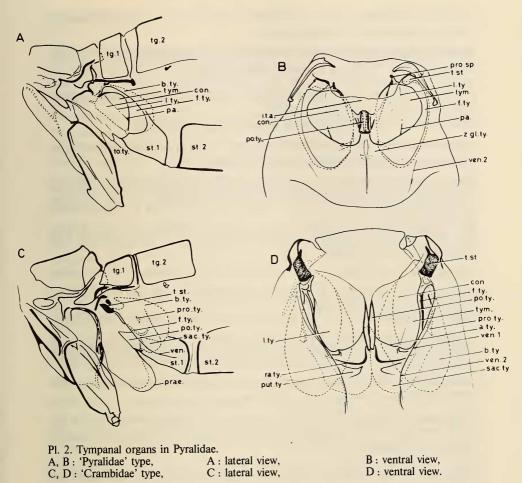
A: Dolicharthria punctalis (Den. & Schiff.) ventral view.

B: Titanio normalis (HBn.), ventral view.

C: Pyrausta cingulata (L.), frontal view.

D: Nomophila noctuella (Den. & Schiff.), ventral view.

E: Pyrausta cingulata (L.), ventral view.



B: ventral view, D: ventral view.

ABBREVIATIONS

a.ty.	ala tympani	put.ty.	puteolus tympani
b.ty.	bulla tympani	ra.ty.	ramus tympani
con.	conjunctivum	ru.od.	rugae odontoidae
f.ty.	fornix tympani	sac.ty.	saccus tympani
i.t.a.	intersegmental thoraco-abdominal	sco.	scoloparium
	membrane	spi.	spinula
l.ty.	ligna tympani	t.st.	tergo-sternal sclerite
pa.	paraspina	to.ty.	torulus tympani
po.ty.	pons tympani	tym.	tympanum
prae.	praecinctorium	ven.	venula
pro.sp.	processus spiniforme	z.gl.ty.	zona glabra tympani
pro. tv.	processus tympani		

It is more developed in the 'Crambidae' type were it is found as a larger simple or bilobed sac: praecinctorium.

processus spiniforme (processus spiniforme): small protuberance on the anterior part of the bullae tympani (closed forms).

processus tympani (saillie tympanique): invagination of the bulla tympani beneath or even in the fornix tympani.

When present it is the attachment place for the scoloparium.

rugae odontoidae (stries odontoïdes) : small wrinkles on the margin tympanum-fornix tympani, restricted to the 'Pyralidae' type.

saccus tympani (poche tympanique and fossette tympanique): In certain 'Crambidae' the fornix tympani is strongly invaginated in the sternite, forming a cavity.

This cavity can be strongly developed posterior to the fornix tympani, thus forming the saccus tympani.

scoloparium (scoloparium): the chordotonal nerve consisting of four scolopale cells and connected between the tympanum and the dorsal or dorsolateral side of the bulla tympani.

When the organs are dry it can be seen through the transparent tympanum.

spinula (spinula): small sclerite on the inside of the tympanum marking the attachment place of the scoloparium, usually conical, sometimes plate-like.

tergo-sternal sclerite (tergite tergo-sternal): small sclerite anterior to the fornix tympani and between the tergite and sclerite.

tympanum (tympanum): very thin membrane, transparent when dry, of the auditory organ. The chordotonal sensilla are directly or indirectly, through a spinula, attached to it.

venula, pl. venulae (venula): medio-lateral sclerotized bar in the inside of the sternite.

Two forms are recognized:

- 1) venula prima, pl. : venulae primae : lateral to the fornix tympani, usually only a fold in the pleural membrane
- 2) venula secunda, pl.: venulae secundae: posterior to the fornix tympani, usually starting as an extension of the venula prima at the ramus tympani. More strongly sclerotized as the venula prima.

zona glabra tympani (dépression tympanique): zone posterior to the pons tympani and the rami tympani devoid of scales, sometimes sloping and then forming a puteolus tympani (pl. puteoli tympani).

Methodology

Slides should be made in such a way that all characters are preserved and displayed, suitable for drawing or photography. The slides must be permanent and in such a form that they may be examined repetitively without damage. These standards, as put forward by Robinson (1976), are also valid for tympanal organs. The preparation technique proposed here is principally an adaptation of the methods proposed by Robinson (I.c.). Only here, the abdomen is mounted under a separate cover slip beside the genitalia. Two plastic rods are used to prevent flattening and distortion of the tympanal organs (the plastic must be tested to ensure it will not dissolve in Euparal Essence; use f.i. Teflon). Contrary to Minet (1983), the praecinctorium, when present, is left on the metathorax. When left on the pons tympani, the praecinctorium is difficult to clean and gets easily distorted, resulting in the loss of a character. Also it covers major parts of the zona glabra tympani and obscures details which offer more important characters as the praecinctorium.

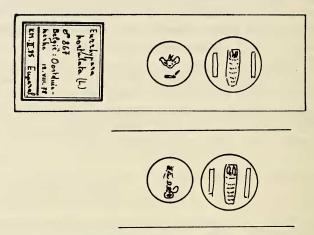
For the preparation of these structures a dissection microscope is necessary. For studying them, a mono- or binocular microscope is strongly advised.

A flattened needle with a sharpened side is used to make a small cut posterior to the praecinctorium, anterior to the pons tympani. The abdomen is then easily broken off by a small pressure on the ventral side. Once broken off the abdomen is boiled in 10% KOH. Afterwards it is dissected in 50% ethanol using bent micropins and/or snipe feathers. By using the latter, ruptures in the abdomen are avoided. They are very useful to brush off the scales. Before extracting the genitalia, staining in Chlorazol Black E (1% solution in 70% ethanol) is necessary to make the intersegmental membranes more visible. The abdomen should be left only a few seconds in Chlorazol Black. During the dissection several stainings are preferred to just one since overstaining is very difficult to reduce.

At this point a small syringe (1 ml) with a blunt needle can be used. Holding the abdomen, tergites ventrally, with the snipe feather on sternite one, the syringe is placed just in front of the anterior end of the abdomen and ethanol is squirted into it. This way most of the chitinous membranes leave the abdomen and distorted bullae tympani are restored to the correct shape. What remains can be extracted with the bent micropins. During the dissection great care should be taken to keep the tympanum, eventually the spinula, and the conjunctivum intact.

Once the staining is satisfactory, it is fixed in ethanol 96%. After complete dehydration the structures are transferred to Euparal essence. The genitalia

are then mounted in Euparal in the middle of a slide (δ : valva spread; aedoeagus horizontal, under the genitalia, apical end to the left; \mathfrak{P} : orientated along the ventral axis of the slide, papillae anales to the top) and covered with a cover slip of appropriate size. The abdomen is mounted parallel to the genitalia ventral side uppermost and tympanals to the top. Longer abdomens are orientated along the horizontal axis of the slide.



Pl. 3. Labelling and slide arrangement for Pyralidae

As already mentioned small plastic strips are used to support the cover slip. Plastic with a thickness of 0,5 mm was found most suitable since it can be used for most Pyralidae, except Siginae, Midilinae and some others. For these groups the abdomen itself is sclerotized enough to support the cover slip. The plastic strips merely prevent a strong inclination of the cover slip. An oblique cover slip distorts the image under the microscope. To develop a standard method, 0,5 mm is strongly advised.

After mounting and labelling, the slides are kept flat and preferably transferred to an oven at 45° C. Air bubbles disappear easily but also the Euparal essence evaporates. The slides have to be checked regularly and Euparal added. The first time after a few hours, followed by once a day the following week. Afterwards they can be stored in a slide cabinet although a last check after a few months is advisable.

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